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Influence of Some Technological Factors on the Preparation of Polymeric Nanoparticles with Indomethacin

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Abstract

During the past decade research defines nanoparticles from biocompatible and biodegradable polymers as efficient systems for drug delivery in the human organism. This is relevant for practically insoluble drugs in water, whose application in liquid forms (eye solutions for example) is hindered and not effective enough.

The aim of the work was to present the preparation of model poly (vinyl acetate) nanosupports for indomethacin (IMC) by a radical polymerization of the monomer in the presence of IMC and without an emulsifier. In this case we investigated the influence of the various methods for releasing nano particles from low molecular weight compounds (initiator, residual monomer or free IMC) and the effects of ultrasonic stirring during the time of polymerization. The nanoparticles characterized with SEM, TEM and FTIR analysis showed a size around 200 nm. The release of IMC from the nanoparticles was evaluated in pH 7.4 and showed a delayed release of the drug compared to the pure IMC from those carriers which are not chemical interaction between the drug and polymer. There wasn`t any drug releasing from the carrier with the eventual stable complex formation between the polymer and IMC.

This investigation showed that conditions of polymerization and the subsequent method of elimination of low molecular weight compounds are essential for the morphology and the release of the included IMC.

Keywords: Nanoparticles; Radical polymerization; Indometacin; Poly(vinyl acetate); Ultrasonic stirring; Morphological characterization; Residual monomer

Abbreviations: IMC: Indomethacin; SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; FTIR: Fourier Transform Infrared Spectroscopy

Introduction

Indomethacin (IMC), ([1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid) is a nonsteroidal anti-inflammatory drug [1]. It is practically insoluble in water, unstable in alkaline and acidic media and slightly soluble in alcohol [2,3]. Due to its insolubility in water, the drug formulations, in which it is included, often show low and erratic bioavailability, and in oral use there is an increase in irritation of the lining of the stomach, caused by prolonged contact with it [4].

In ophthalmology, IMC is used as topical eye drops for prevention of miosis during cataract surgery, cystoid macular edema and conjunctivitis [5,6]. Its use in liquid formulations is limited due to its properties.

During the past decade, research defines the use of Nanoparticles (NPs) from biocompatible and biodegradable polymers as an effective drug-release system, whose aim is to increase the solubility, with consequent increase of the bioavailability and reduction of the irritating effects of the drug [6]. The choice of a polymer, method and technological factors has a crucial role for the degree of drug loading, stability and its dissolution rate.

Different possibilities about polymer choice and IMC loading methods are discussed in the literature. For example NPs, based on copolymers of methyl methacrylate and glycidyl methacrylate with IMC were developed, via emulsion radical polymerization [7].

Moreover, some studies were already made on NPs containing cyclodextrins and IMC [8].

Poly(ε-caprolactonic) NPs, nanocapsules and nanoemulsions of IMC were obtained, (average size 225 nm), via superficial cumulation, nanoprecipitation and spontaneous emulsification [9].

In another study Rezaei Mokarram et al. [10] established that the enhanced solubility and dissolution rate of IMC compared to physical mixtures and crystalline form of IMC (polymorph I), mean that it interacts with poly(vinyl pyrrolidone) via hydrogen bond and probably forming eutectic mixture [10].

The aim of this work was to prepare of model poly (vinyl acetate) (PVAc) nanosupports for IMC by a radical polymerization of the monomers in the presence of the drug, without an emulsifier, and to study the influence of various methods for releasing of NPs from low molecular weight compounds (initiator, residual monomer or free IMC) and the effects of ultrasonic impact during the time of polymerization.

Materials and Methods

In this research, the following materials were used: Indomethacin, puriss ≥ 99.0%, Fluka BioChemika; Potassium dihydrogen phosphate pro analysis, Merck; Sodium dihydrogen phosphate pro analysis, Merck; Vinyl acetate, puriss. ≥ 99.5% (GC), Fluka; Ammonium per sulfate, purum, pro analysis, ACS reagent, ≥ 98% (RT), Fluka.

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The poly (vinyl acetate) nanoparticles (IMC-PVAc) were made by a radical polymerization of vinyl acetate (VAc), in the presence of IMC (1%, w/v) and monomer (10%, v/v). The polymerization was conducted in a nitrogen atmosphere and at a temperature of 50°C, for 90 min. Ammonium persulphate (AP) in concentration 1% (w/v) was used as initiator. In our previous work, applying the same method of polymerization, we show the effect of ultrasound impact during the time of polymerization, which is expressed in the realizing of IMC from nanosized latex [11]. In a following work we investigate the influence of dialysis as a method for elimination of the low molecular weight compounds (initiator, residual monomer or IMC) from the primary latex [12]. The model latex is exposed to dialysis for 6, 9, 18 and 23 h. The investigation shows that the models which are dialyzed for 9, 18 and 23 h contain more IMC in comparison with the model which is dialyzed for 6 h. In the current study we combined the methods of the both previous works. We increased the time of dialysis up to 50 h and we applied one more method for elimination of the low molecular weight compounds. The primary latex was dried at 60°C then washed with water and acetone and was further dried at the same temperature. The resulting film after drying was easily crushed to powder, consisting of raw latex nanoparticles. Thus we defined the methods:

Method A – polymerization with subsequent dialysis for 50 h.

Method B – polymerization with subsequent drying at 60°C then washing with water and acetone and new drying at the same temperature.

Method 1 – polymerization without ultrasound stirring.

Method 2 – polymerization with ultrasound stirring.

The Scanning Electron Microscopy (SEM)

SEM was made at JEOL JSM-5510 at 10 kV, using a device for cathodic pulverization and application of thin layers of gold (Fine Coater JEOL JFC-1200).

The Transmission Electron Microscopy (TEM)

TEM scan to the investigated models is held on transmission electron microscope JEOL JEM 2100 acceleration voltage 200 kV. Micro - quantities of the test substance is mixed in a test tube with distilled water. Placed in an ultrasonic bath for 3 min and immediately thereafter, with pipette, suspension is dripped on pre-coated with carbon standard Cu grid. After air-drying in a dust free environment for several hours the grid is ready for observation in the microscope.

Fourier Transform Infrared (FTIR)

FTIR spectroscopic analyses were carried out with FTIR Bruker Tensor 37 Spectrometer, using the technique of tableting with KBr and Resolution 2 cm^{-1} at 120 scans for each sample.

Examination on the release of IMC

Examination on the release of IMC from the model NPs was carried out in sink conditions in a termostated vessel with equal amounts of the tested models, working volume for dissolution 100.0 ml phosphate – phosphate buffer (Sorensen's phosphate buffer) at pH 7.4; temperature 37°C; stirring speed 100 min^{-1} [11-13]. At appropriate time intervals suitable aliquots were withdrawn and replaced with fresh buffer. The quantitative defining of IMC was made spectrophotometrically at λ=320 nm on UV/VIS spectrophotometer Ultrospec 3300 pro after filtering the samples through a filter Chromafil Xtra 0.45 μm. The measurements were made compared to the middle of examination Sorensen's phosphate buffer at pH 7.4. Each survey was conducted 6 times. Results are expressed as percentage of total drug loading.

Results and Discussion

The conversion rate on the monomers was 97-98% during the time of the polymerization (90 min). Table 1 shows the studied model NPs and the method of their obtaining. Surfactants were not used during the process of polymerization or NPs testing.

Results from SEM

Model IMC-PVAc-1B was subjected to SEM. It shows an approximate particle size about 200 nm for the observed model (Figure 1).

Results from TEM

Figure 2 presents TEM – pictures of the examined models. They show the average size of the particles, which is about 200 nm, and their round shape. On four of the models, we can observe a structural change, which is an indicator for a process of crystallization. It can be explained with the presence of IMC, as a crystal substance. Especially indicative is model IMC-PVAc-2B, where the particles of IMC has crystallised over the surface of the carrier. At model IMC-PVAc-1B IMC is observed both on the surface, and inside the nano particle. Models IMC-PVAc-1A and IMC-PVAc-2A show denser structures. In our previous mentioned study we assumed that during the time of the dialysis are formed pores in the PVAc matrix [12]. The more complete

Designation	Method combinations
IMC-PVAc-1B	Method 1 and Method B
IMC-PVAc-1A	Method 1 and Method A
IMC-PVAc-2B	Method 2 and Method B
IMC-PVAc-2A	Method 2 and Method A

Table 1: Investigated nanosized particles with IMC.

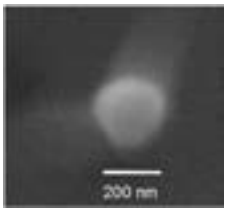

Figure 1: Scanning electron microscopy of nanosized particles of IMC-PVAc-1B.

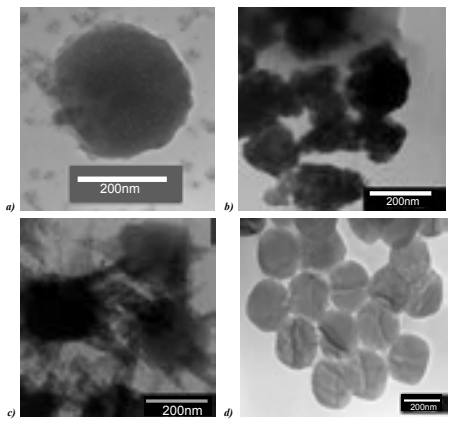

Figure 2: TEM at a) IMC-PVAc-1B, b) IMC-PVAc-1A, c) IMC-PVAc-2B and d) IMC-PVAc-2A.

the extraction of the monomer and the initiator from the matrix is, the more pores are formed, which allows a complete release of IMC incorporated in the matrix. After 9 hours of dialysis, the extraction of the residual monomer and initiator was complete, no more pores were formed and the amount of released IMC became independent from the time of dialysis. At the time of the dialysis between 23 and 50 h apparently occur chemical changes where the weak hydrogen bonds between the polymer and IMC in the models which were dialyzed less time from 23 h are probably converted into stable chemical bonds. Possible reason for this interaction is the liquid medium in which the IMC-PVAc-NPs are located. In previous study [11] we showed the presence of chemical bonds between IMC and polymer in primary latex, before it is undergone any other technological treatment. This could be a probable reason for their more dense structure compared to the models which were obtained by combination of Methods 1 and 2 with Method B.

FT-IR results

Figure 3 shows the IR-spectrum of the four models. In the spectra of pure IMC (γ – type, more stable and less soluble polymorph modification of the drug in comparison with α- modification) are shown two most intensive peaks at 1717 cm-1 and at 1690 $cm^{-1}$of υC=O [10]. Spectra of models IMC-PVAc-1B and IMC-PVAc-2B (Figure 3a) show a similarity with this of pure IMC (not showed) [10,12,14,15]. Obviously, the current systems IMC-PVAc-1B and IMC-PVAc-2B are not about a chemical interaction between polymer and IMC but rather for an interaction with hydrogen bonds. At IMC-PVAc-1A and IMC-PVAc-2A (Figure 3b) we see some of the characteristic absorption peaks, but we must probably look for a complex between the drug and the monomer due to a lack or a shifting of peaks that are typical for the pure IMC [10-12,14,15].

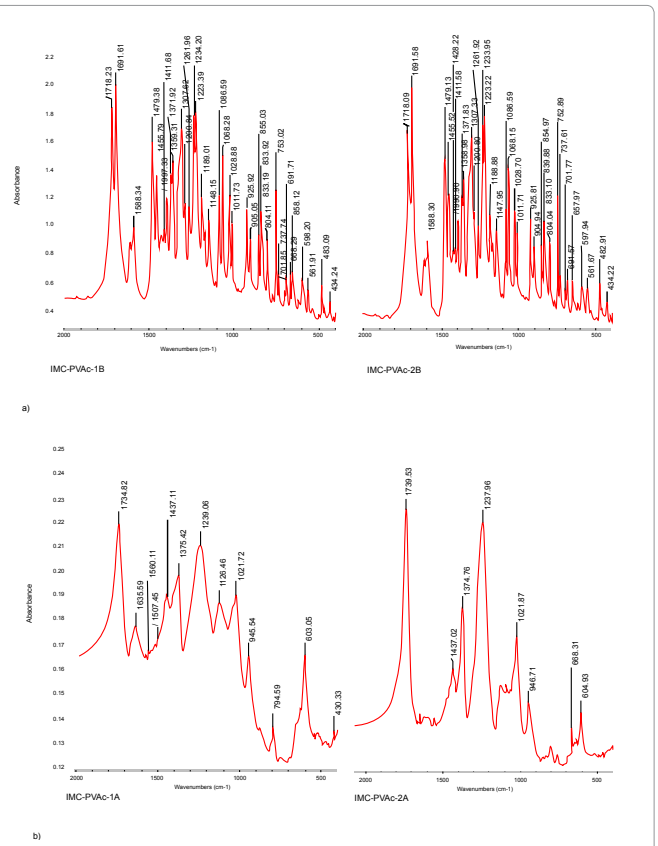

Figure 3: IR-spectra: at a) IMC-PVAc-1B and IMC-PVAc-2B; at b) IMC-PVAc-1A and IMC-PVAc-2A.

Examination of the release of IMC from model carriers in phosphate-phosphate buffer with pH 7.4

Study of the drug release from IMC-PVAc-NPs showed logarithmically increase of the IMC concentration with time at model IMC-PVAc-1B and IMC-PVAc-2B (Figure 4). For the same time of examination, model IMC-PVAc-2B released faster and in greater degree the included IMC, reaching above 90% of NPs drug loaded unlike of IMC-PVAc-1B which dissolution rate reached 80% of included IMC. A probable reason for this may be the ultrasound impact, during the process of polymerization, where the IMC crystallize over the surface of the nanoparticle, especially in combination with Method B. Both models showed delayed dissolution rate compared to those of pure IMC profile (not showed) [10-13,16].

Models IMC-PVAc-1A and IMC-PVAc-2A didn't show any release of IMC in the middle of examination. It is probably due to the applied dialysis, as well as the obtaining of stable complexes under the conditions of dialysis for 50 h and liophilization – a problem which is not forthcoming to our previous study [12].

As it was mentioned in our previous study, we found that exposure of primary latex on dialysis for 9, 18 and 23 h showed no significant differences in the amounts of the IMC and its release from the polymer particle as opposed to the model, which was dialyzed 6 h [12]. The increased time of dialysis of the models up to 50 h led to NPs with dense structure and probably to chemical complex between the polymer and the drug. At the time of dialysis between 23 and 50 h apparently occur chemical changes where the weak hydrogen bonds between the polymer and IMC are probably converted into stable.

So, time of dialysis for 50 h was inappropriate for the preparation of IMC-PVAc-NPs. The application of Method B (polymerization with subsequent drying at 60°C then washing with water and acetone and new drying at the same temperature) in combination with Method 1 and Method 2 led to alteration of the NP such as in morphology, entrapped IMC and profiles release. Considering that IMC is practically insoluble in water, applying of ultrasound impact during the polymerization possibly leads to better homogenization of the drug, respectively a greater amount of its incorporating with weak hydrogen bonds. In this regard the combination of Method 2 and Method B is preferable to that of Method 1 and Method B.

The study showed that the type of the method of purification of

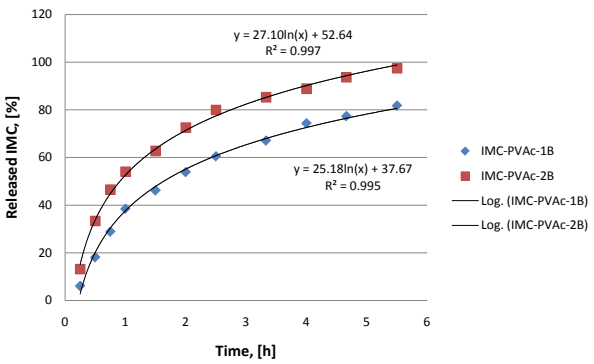

Figure 4: Profiles of release of IMC from models IMC-PVAc-1B and IMC-PVAc-2B.

the NPs from the low molecular weight substances in combination with applied sonication is essential for the morphology of the NPs and the state of IMC which is incorporated therein.

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